QIAAMP DIFFERENTIAL EXTRACTION

A. SCOPE

This protocol employs the QIAamp DNA Mini Kit, a digest buffer, and dithiothreitol (DTT) for the extraction of differentially separated epithelial and sperm fractions.

B. QUALITY CONTROL

B.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure.

B.2 Each new QIAamp DNA Mini Kit lot must undergo quality control testing prior to extracting casework samples.

Biological material with known results along with a reagent control will be extracted using all the components of the kit undergoing quality control testing. The extracted material will be carried though the entire DNA analysis process. The results obtained from the known extracted sample must be as expected and good quality, as described in the GlobalFiler interpretation guidelines (DOC ID 12628), for the kit to pass quality control testing. The quality control data will be placed into the critical reagent binder.

B.3 An analyst that dilutes the concentrated Buffers AW1 and AW2 prior to their initial use will be watched by a second individual from the Biology Unit to confirm correct preparation; this second individual can be another analyst, an Investigative Assistant, etc. Both individuals will initial the bottle. In addition, the lot number and expiration date of the added ethanol will be recorded on the bottle.

B.4 See DOC ID 1835 to determine reagent expiration dates.

B.5 Digest buffer and DTT will undergo quality control testing prior to being used in the differential extraction of casework samples.

Samples known to contain a mixture of sperm and epithelial cells with known results along with a reagent control will be extracted using the digest buffer / DTT undergoing quality control testing. The extracted sperm and epithelial fractions will be carried through the entire DNA analysis process. The Digest buffer / DTT will pass quality control testing when a good quality DNA profile, as described in the GlobalFiler interpretation guidelines, is obtained with the expected results for each fraction. The quality control data will be placed into the critical reagent binder. If the same lot number of powder DTT as previously quality control tested needs to be prepared, the analyst preparing the new batch of DTT will be watched by a second individual from the Biology Unit to confirm correct preparation. Both individuals will initial the box. No additional quality control testing is necessary.

B.6 At least two reagent controls must be extracted along with a set of questioned samples.

B.7 When consuming a sample and the corresponding extract, you must keep the post extraction substrate (refer to the DNA Quality Manual, DOC ID 1833 for details on evidence consumption and retention).

C. SAFETY

C.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn if this procedure is performed outside of a hood.

C.2 The sample preparation waste contains guanidine hydrochloride from Buffers AL and AW1, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with water or ethanol.

C.3 All appropriate SDS sheets must be read prior to performing this procedure.

C.4 Treat all biological specimens as potentially infectious.

C.5 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 QIAamp DNA Mini Kit

D.1.1 Buffer AL
D.1.2 Proteinase K
D.1.3 Buffer AW1

Before using for the first time, add 125 mL ethanol (Absolute) to 95 mL AW1 concentrate.

D.1.4 Buffer AW2

Before using for the first time, add 160 mL ethanol (Absolute) to 66 mL AW2 concentrate.

D.1.5 Buffer AE

D.2 Absolute Ethanol (200 proof)

D.3 DTT, 1M Dithiothreitol, 5 mL

Dissolve 0.77 g dithiothreitol in 5 mL sterile deionized water in a sterile, disposable plastic 15 mL tube or original container, or add 6493 µL of deionized water to the bottle containing 1 g of the dithiothreitol. Store 110 µl aliquots in 0.5 mL microcentrifuge tubes at approximately -20° C. Discard any unused portion of a thawed tube.

D.4 Digest Buffer

10Mm Tris-HCl - 10mM EDTA - 50mM NaCl - 2% SDS, pH 7.5, 500 mL

Add 5mL 1 M Tris-HCl (pH 8.0), 10 mL 0.5 M EDTA (pH 8.0), 5 mL 5 M NaCl, and 100 mL 10% SDS to 380 mL deionized water (or use 0.29 g NaCl, e.g. Sigma S-3014 molecular biology grade, in place of the 5 mL 5 M NaCl and adjust volume to 500 mL). Store at room temperature.

D.5 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

D.6 70% Reagent Alcohol (Decontamination)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

- E.1.1 Scissors/Forceps
- E.1.2 Microcentrifuge
- E.1.3 Eppendorf ThermoMixer
- E.1.4 Eppendorf Smartblock 1.5 mL
- E.1.5 Eppendorf Smartblock 2.0 mL
- E.1.6 Eppendorf ThermoTop
- E.1.7 Pipettes
- E.1.8 Vortexer

E.2 Supplies

- E.2.1 Kimwipes
- E.2.2 Microcentrifuge tubes
- E.2.3 Spin baskets
- E.2.4 Sterile aerosol resistant pipette tips
- E.2.5 Microcentrifuge tube racks
- E.2.6 Permanent marker
- E.2.7 Mask
- E.2.8 Lab coat

E.2.9 Disposable gloves
E.2.10 Eye protection (e.g. safety glasses, face shield)
E.2.11 Differential Extraction form

F. PROCEDURES

F.1 Cut the sample and place into a labeled microcentrifuge tube that will hold a spin basket. Add 500 µL Digest Buffer and 15 µL Proteinase K.

F.2 Incubate at approximately 56°C with approximately 550 rpm mixing for at least 1 hour and no more than 2 hours using a ThermoMixer. Lyses time will vary depending on the size and density of the source material. This incubation time must be recorded and can be documented as a start and end time or total incubation time.

Perform step F3 **or** F4 to obtain epithelial and sperm fractions

F.3 Briefly centrifuge the sample to remove drops from inside the lid. Using a pipette tip or sterile forceps remove substrate and place in a spin basket. Place basket back into collection tube and centrifuge for 5 minutes at maximum speed to collect any fluid remaining in substrate. Carefully remove all but approximately 50 µL of the supernatant to a new labeled microcentrifuge tube. This is the non-sperm cell fraction (epithelial cell fraction). Store the sample until the sperm cell digestion is completed. Alternatively, steps F.10 thru F.20 may be performed on the epithelial cell fraction during the sperm cell incubation (step F.9).

F.4 Briefly centrifuge the sample to remove drops from inside the lid. Using a pipette tip or sterile forceps remove the material while compressing it against the tube wall to eliminate any liquid. Centrifuge the sample for 5 minutes at maximum speed. Carefully remove all but approximately 50 µL of the supernatant to a new labeled microcentrifuge tube. This is the non-sperm cell fraction (epithelial cell fraction). Store the sample until the sperm cell digestion is completed. Alternatively, steps F.10 thru F.20 may be performed on the epithelial cell fraction during the sperm cell incubation (step F.9).

F.5 Add 500 or 1000 µL Digest Buffer to the sperm cell pellet. Vortex and centrifuge for 5 minutes at maximum speed. Discard all but approximately 50 µL of the supernatant.

F.6 Repeat step F.5 two to four more times, for a total of 3-5 washes in Digest Buffer.

F.7 (OPTIONAL) Add 500 µL of DI water to the sperm cell pellet. Vortex and centrifuge for 5 minutes at maximum speed. Discard all but approximately 50 µL of the supernatant.

F.8 (OPTIONAL) Re-suspend the pellet in the remaining 50 µL of liquid. Remove 3 µL (or approximately 10% of the sample) of re-suspended cells and place on a slide. Perform Christmas Tree staining and examine microscopically for the presence of spermatozoa (document 1600).

F.9 Add 500 µL Digest Buffer, 15 µL Proteinase K and 20 µL DTT to the tube. Incubate at approximately 56°C with approximately 550 rpm mixing for at least 1 hour and no longer than 24 hours using a ThermoMixer. This incubation time must be recorded and can be documented as a start and end time or total incubation time.

F.10 Briefly centrifuge to remove drops from inside the lid. Add 500 µL AL Buffer to one or both lysed cell fractions (sperm and/or epithelial). Incubate at approximately 56°C with approximately 550 rpm mixing for 10 minutes using a ThermoMixer.

F.11 Briefly centrifuge to remove drops from inside the lid. Add 525 µL of Ethanol (200 proof). Vortex vigorously.

F.12 Briefly centrifuge to remove drops from inside the lid. Carefully remove liquid from the microcentrifuge tube and add to the labeled QIAamp spin column without wetting the rim. Close the cap and centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.13 Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate.

The spin column holds up to 700 µL. Repeat steps F.12 and F.13 as needed.

F.14 Carefully open the spin column and add 500 µL AW1 Buffer without wetting the rim. Close the cap and centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.15 Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate. Carefully open the spin column and add 500 µL AW2 Buffer without wetting the rim. Close the cap and centrifuge at approximately **20,800 rcf (14,000 rpm)** for 3 minutes.

F.16 Continue with step F.18, or if there is AW2 Buffer carryover, perform step F.17.

F.17 (Optional): Place the QIAamp spin column in a new collection tube and discard the collection tube with the filtrate. Centrifuge at approximately **20,800 rcf (14,000 rpm)** for 1 minute.

F.18 Place the QIAamp spin column in a clean labeled microcentrifuge tube (this will be the final storage tube) and discard the tube containing the filtrate. Carefully open the spin column and add 50 to 200 µL of AE Buffer.

F.19 Incubate at room temperature for at least 1 minute.

F.20 Centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.21 Quantitate (DOC ID's 1784 and 1785) the DNA and concentrate samples (DOC ID 1780) as necessary. Alternatively, samples may be concentrated prior to quantitation. Store sample extracts in the refrigerator when not in use. Sample extracts may be frozen for long-term storage.

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 QIAamp DNA Mini and Blood Mini Handbook. Third Edition, 04/2010